REMARKS/ARGUMENTS

Claims 23, 26-28 and 33, and 35-42 are active in this application.

Claim 23 is amended to define the specific L-amino acids produced by the claimed method as previously presented in Claim 25 and 26-28.

Claims 26-28 have been amended to change their dependencies based on the amendment to Claim 23 and the cancellation of Claim 25.

No new matter is added.

The Examiner has also maintained the obviousness rejections citing primarily to Kikuchi, Shimizu, and Chang. The Matsui and Dunican citations are for aspects of the certain dependent claims. The reasoning of the rejection is as follows.

Kikuchi teaches producing L-amino acids in *E. coli* but not the inactivation of the poxB gene as in the claims. Shimizu teaches that acetate has a negative effect on cell growth and Chang teaches that poxB catalyzes acetate from pyruvate, it would have been obvious to inactive the poxB gene to minimize the production of acetate thereby increasing the production of L-amino acids.

Applicants respectfully disagree with the findings of fact and conclusion based thereon that give rise to each of the rejections.

Shimizu discloses that the cell growth inhibiting substances can be removed by controlling the acetate concentration so as to be at most 17g/l (Col. 4, lines 44-50). Therefore a concentration above 17g/l acetate inhibits the growth of *E. Coli* bacteria. So it is not true that Shimizu teaches generally that acetate inhibits growth of E. coli in culture, as indicated in the rejection.

Chang, however, discloses that the feeble growth of modified bacteria is the result of the modification by inactivation of the pyruvate dehydrogenase complex by deletion of aceFE

genes, so that the cells use the (energy consuming) pathway using the pyruvat oxidase complex (poxB). According to Chang this results in a worse growth with acetate, because these mutations lead to cells which are only able to grow with the acetate which is generated with poxB (page 757, right col. bottom).

This phenomenon has nothing to do with an inactivation of cell growth by acetate. So Chang in fact does not *teach* that *acetate inhibits growth of E. coli in culture* as the examiner has generally stated in the Office Action cited above. Therefore there was no motivation for those skilled in the art to combine Shimizu and Chang. Such a combination is a result of a hindsight approach.

Chang further says that *it remains unclear why the growth of E. coli on the acetate produced by pyruvate oxidase is so feeble* and *that the role of these pathways in utilization of the acetate produced by pyruvate oxidase is under investigation* (page 762 last paragraph) which also indicates that the consequences of the genetic modifications were not predictable due to the complexity of biochemical regulation mechanisms.

Furthermore Chang does not use L-amino acid production strains but (modified) natural strains, which means that it cannot be concluded from alterations in natural strains which production relevant results might come out while making the same alterations in production strains.

The Examiner argues that because Chang teaches the role of poxB in producing acetate, inactivating poxB blocks acetate production and Shimizu teaches that acetate inhibits bacterial growth at some concentrations, one would have inactivated poxB in Kikuchi or Matsui's bacteria leading to a reasonable predictability of successfully producing a strain that would produce L-amino acids. Applicants respectfully disagree.

Indeed, the pathway in which poxB is involved is not the primary source of acetate in a cell. As explained in the attached Chang et al (1999) J. Bacteriol. 181 , 6657 (Results):

> *Introduction of a pta mutation did not completely eliminate acetate production during the growth phase. The acetate yield of the pta mutant on glucose, 0.11 mM z (g [dry weight])21, was reduced to one-fourth that of the wild type, 0.45 mM z (g [dry weight])21. Pyruvate oxidase (PoxB) presents a possible route for the generation of acetate in the pta mutant (Chang, Y.-Y., A.-Y. Wang, and J. E. Cronan, Jr. 1994. Expression of Escherichia coli pyruvate oxidase (PoxB) depends on the sigma factor encoded by the rpoS (katF) gene. Mol. Microbiol. 11:1019–1028.), because acetyl phosphate may be converted to acetate and inorganic phosphate through the action of either AckA or nonenzymatic degradation (Brown, T. D. K., M. C. Jones-Mortimer, and H. L. Kornberg. 1977. The enzymatic interconversions of acetate and acetyl-coenzyme A in Escherichia coli. J. Gen. Microbiol. 102:327–336.). However, a further mutation in the poxB gene did not result in the complete elimination of acetate (Shin, S., and J. G. Pan. unpublished data.).*

Therefore, it becomes clear that the pathway involving poxB is not the primary source for acetate. Many other sources can be found for amino acid or fatty acid metabolism as shown in the attached overview of the KEGG-Pathways (enclosed)).

It was generally known that acetate may significantly lower yield and productivity in different processes involving microorganisms. 9 g/l of acetate cause inhibition of cell growth (Xu et al., Applied Microbiology and Biotechnology, Volume 51, Number 5 / May 1999, page 564-571). This is not a particular problem of L-amino acid production.

Therefore, one who wanted to solve the problem of increasing the yield in fermentation processes for making L-threonine, L-valine or L-lysine would not have specifically sought to lower acetate formation and certainly not the inactivation of poxB. Therefore, contrary to the conclusion in the Action, which is clearly based on hindsight, the combination of art does not lead one to inactivate poxB in Kikuchi or Matsui's bacteria

leading to a reasonable predictability of successfully producing a strain that would produce the L-amino acids as defined in the claims.

Accordingly, the rejections applied are not tenable. It is requested that the rejections under 35 USC 103(a) citing (A) Kikuchi, Shimizu and Change; (B) references in (A) further in view of Matsui; (C) references in (A) further in view of Dunican be withdrawn.

To the rejections under the doctrine of obviousness-type double patenting (provisional or otherwise):

10/812,315 is abandoned and therefore no further action is deemed to be required.

11/658,477 has issued as 7,759,094. A Terminal Disclaimer over this patent and 7,504,242; 6,759,218 and 7,205,131 is attached to this paper.

10/847,610 is pending and as stated in MPEP § 822.01: If the "provisional" double patenting rejection in the present application is the only rejection remaining, the examiner should then withdraw that rejection and permit the present application to issue as a patent, thereby converting the "provisional" double patenting rejection in the other application(s), if applicable, into a double patenting rejection at the time the present application issues as a patent.

Should the Examiner wish to discuss any aspect of this application, he is invited to contact the Applicants' undersigned representative.

Respectfully submitted,

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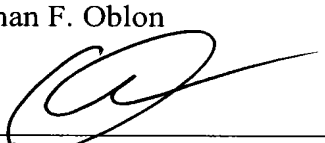

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